Life-history trait variation in a queen-size dimorphic ant

Wolf, Jana I.

2018-12

Wolf , J I , Punttila , P & Seppä , P 2018 , ' Life-history trait variation in a queen-size dimorphic ant ' , Ecological Entomology , vol. 43 , no. 6 , pp. 763-773 . https://doi.org/10.1111/een.12662

http://hdl.handle.net/10138/323997
https://doi.org/10.1111/een.12662

acceptedVersion

Life-history trait variation in a queen-size dimorphic ant

Jana Irina Wolf[1,2], Pekka Punttila[3] and Perttu Seppä[1,2,4]

[1]Centre of Excellence in Biological Interactions, Organismal and Evolutionary Biology Research Program, Faculty of Biological and Environmental Sciences, P.O. Box 65, FI-00014 University of Helsinki, Finland

[2]Tvärminne Zoological Station, University of Helsinki J.A. Palménin tie 260, FI-10900 Hanko, Finland

[3]Finnish Environment Institute, P.O. Box 140, FI-00251, Helsinki, Finland

[4]Corresponding author: Organismal and Evolutionary Biology Research Program, Faculty of Biological and Environmental Sciences, P.O. Box 65, FI-00014 University of Helsinki, Finland, perttu.seppa@helsinki.fi

Running title: Life-history variation in size-dimorphic ants

ABSTRACT

1. Size polymorphism is often connected to alternative life-history traits, which may eventually lead to distinct size classes. In the ant *Myrmica ruginodis*, larger macrogyne and smaller microgyne queen morphs have been suggested to follow different reproductive strategies, which has presumably resulted in several differences in their key life-history traits.

2. In this study, we examine the association of queen-size morphs with colony queen number (monogyny vs. polygyny), dispersal and queen recruitment patterns as well as habitat associations of the queen morphs. We do this by sampling established queens from a large number of excavated nests from several populations, estimating genetic relatedness among coexisting queens and pitfall trapping free-ranging wingless queens.

3. Our results show that associations of queen morphs with colony queen number and nest-founding strategy holds only partly. The morph frequencies vary widely across populations from practically pure macrogyne to more than 50% microgyne, but the expected association of macrogyne occurrence with monogyny and microgyne with polygyny is not universal. Dispersal and queen recruitment patterns also show that while most macrogynes participate in nuptial flights and most microgynes are recruited back to their natal nests, a fraction of both morphs use the alternative strategy.

4. Polygynous microgyne morph has been suggested to specialize in stable habitats, but our results from Finnish mesic heath forests do not support this. This study shows that other factors than just queen size also influence life-history trait variation and reproductive strategies in ants.

Keywords: Ants, Habitat stability, Myrmica, Macrogyne, Microgyne, Size polymorphism, Queen number, Queen recruitment

INTRODUCTION

Alternative reproductive strategies are a widely known phenomenon in the animal kingdom, and they are often associated with intraspecific size variation (Eberhard, 1980; Thornhill & Alcock, 1983; Gross, 1985, 1996; Crespi, 1988; Danforth, 1991). Size often strongly influences fitness, with larger individuals monopolising reproductive opportunities (Roff, 1992; Stearns, 1992). Alternatively, commonly occurring smaller ("sneaker") males seek reproductive opportunities alongside large, dominant and territorially behaving males (Eberhard, 1980; Thornhill & Alcock, 1983; Gross, 1985; Crespi, 1988; Danforth, 1991). Size-dependent strategies can also be connected to dispersal (Dingle *et al.*, 1980; Derr *et al.*, 1981; Gonzaga & Vasconcellos-Neto, 2001), where smaller individuals are usually assumed to be competitively inferior and thus disperse while larger ones behave philopatrically (Lawrence, 1987; Hanski *et al.*, 1991). Nevertheless, this can also be reversed so that larger individuals disperse further than smaller conspecifics due to larger energy reserves (Anholt, 1990; O'Riain *et al.*, 1996; Léna *et al.*, 1998; Gundersen *et al.*, 2002; Barbraud *et al.*, 2003).

In ants, alternative reproductive strategies have been found especially in females, sometimes causing divergence in queen sizes and life-history strategies (Heinze & Tsuji, 1995; Peeters & Ito, 2001). Typically, queens in nests with only a single reproducing queen (monogyny) are larger than those in nests with many queens (polygyny) (see Peeters & Ito, 2001). Large queens have larger glycogen and fat content than small queens, and they are also typically better equipped for dispersing by flight and independent nest founding (Passera & Keller, 1990; Heinze & Hölldobler, 1993; McInnes & Tschinkel, 1995; Wheeler & Martinez, 1995; Stille, 1996; Lachaud *et al.*, 1999). Positive relatedness among coexisting queens in secondarily polygynous species suggests that new queens are often recruited to their natal nests (e.g. Crozier & Pamilo, 1996). Colony propagation occurs then without dispersal by flying, via splitting of old nests (dependent colony founding), which might stay connected (budding) or not (fission) (Rosengren & Pamilo, 1983; Hölldobler & Wilson, 1990). Life-history traits in ants are often described as suites of linked characteristics centred around queen number (so-called polygyny syndrome, Rosengren & Pamilo, 1983).

The occurrence of polygyny in ants as an indicator of reduced dispersal has been associated with stability, patchiness and longevity of the habitat (Hölldobler & Wilson, 1977; Rosengren & Pamilo, 1983), as well as habitat crowding (Herbers, 1986). In stable habitats, patch size sets limits to the growth and propagation of nests through nest-site limitation, resource limitation and competition in general, selecting for increased polygyny (Hölldobler & Wilson, 1977; Rosengren & Pamilo, 1983; Herbers, 1986). If queen dispersal does not exceed further than the natal habitat patch and the number of nests that can co-occur in a given patch is restricted, such habitat patches are expected to become saturated with time with conspecific nests. In such a situation, the only possibility for newly produced queens to reproduce is to be recruited to an existing nest. Interspecific competition and high queen predation during colony founding can also make nest founding more difficult. Thus, both intra- and interspecific competition may reduce nest-founding opportunities, which increases dispersal risk and promotes queen philopatry (Greenwood, 1980) and recruitment.

Sometimes alternative reproductive strategies in ants lead to the emergence of queen-size dimorphism, with either clearly separated or overlapping size distributions between the larger (macrogynes) and smaller (microgynes) queen morphs (Heinze & Tsuji, 1995; Heinze & Keller, 2000; Peeters & Ito, 2001; Wolf & Seppä, 2016a). Size morphs have been suggested to be associated with differences in life-history traits. Macrogyne queens supposedly disperse and are usually found in monogynous nests, which are founded independently after a nuptial flight (e.g. Elmes 1991; Rüppell & Heinze 1999; Wolf & Seppä 2016a). In contrast, microgyne queens do not usually join nuptial flights, as they are poorly equipped for both dispersal (Passera & Keller, 1990; Heinze & Hölldobler, 1993; Wheeler & Martinez, 1995; Stille, 1996; Wolf & Seppä, 2016b) and independent colony founding (McInnes & Tschinkel, 1995; Peeters & Ito, 2001). Instead, they presumably mate locally and seek adoption in secondarily polygynous nests instead.

In ant genus *Myrmica*, there are two queen-size dimorphic species. The microgyne morph of *M. rubra* is a true inquiline parasite (Seifert 1993) currently speciating from its macrogynous host (e.g. Leppänen et al. 2015), whereas in *M. ruginodis* macrogynes and microgynes are free-living intraspecific queen morphs. The size distributions of the two morphs in *M. ruginodis* are overlapping, however, so that not all queens can be

positively assigned to one of the morphs, and about one fourth of the polygynous nests have a mixture of queen morphs (Elmes, 1991).

Since nest-founding strategies in queen-size dimorphic ants may differ depending on habitat quality and patchiness (Lenoir *et al.*, 2011), size morph frequencies are also expected to differ across habitat types. For instance, in *Temnothorax rugatulus*, microgynes have been suggested to profit from stable nesting substrate, such as rocky outcrops, and to occur more often in high densities in those habitats, whereas macrogynes are better colonisers than microgynes (Rüppell *et al.*, 2001). Previous suggestions about the association of *M. ruginodis* queen morphs with the habitat type are somewhat contradictory. On one hand, Brian and Brian (1949) studied *M. ruginodis* in English and Scottish moor habitats and suggested that macrogynes are predominant in transitory habitats ("seral and mosaic cyclic vegetation types") and microgynes are found in relatively stable habitats. On the other hand, our study habitat, boreal forest, is a dynamic mosaic of different successional stages due to various disturbances (Kuuluvainen, 2009; Kuuluvainen & Aakala, 2011). In this type of landscape, nests in early-successional habitats following recent disturbance are more polygynous than in more stable late-successional habitats (Seppä *et al.*, 1995). This suggests that the polygynous microgyne morph specialises in rapidly changing early successional stages rather than relatively stable old-growth habitats.

In this study, we investigated to what extent size morphs in the ant *M. ruginodis* are associated with key life-history traits. For this purpose, we assessed queen-morph identity and its association with i) number of established old queens in excavated colonies, ii) queen dispersal patterns based on temporal distribution of free-ranging wingless queens and iii) queen-recruitment patterns by estimating genetic relatedness among co-existing queens separately in macrogyne and microgyne nests. Studying multiple populations and complementing the results with previously published data (Brian & Brian, 1949; Elmes, 1991; Wolf & Seppä 2016b) enabled us to assess the spatial variation in the occurrence of the queen morphs and their association with colony social structure, dispersal and colony founding patterns.

As suggested earlier (Brian & Brian, 1949; Elmes, 1991), we expected to find a bimodal queen-size distribution in *M. ruginodis* and that macrogyne and microgyne queens are

generally found in monogynous and polygynous nests, respectively. Based on the close association of the colony queen number and other key life-history traits (Heinze & Tsuji, 1995; Peeters & Ito, 2001), we also expect to find that macrogyne queens are more prone to dispersing by flight than microgyne queens. This is expected to show in the pitfall data as an excess of free-ranging wingless macrogyne queens after the nuptial flight and more even distribution of wingless microgynes across the season. Furthermore, we expected to find that coexisting microgyne queens are related, which would indicate that they disperse locally and are recruited back to their natal nests. The exact prediction of queen relatedness in macrogynes is not clear, because macrogyne queens are rarely expected to reside in polygynous nests. Finally, we analyse habitat association of free-ranging wingless queens and test earlier hypotheses (see above) about the occurrence of the monogynous (macrogyne) and polygynous (microgyne) morphs.

MATERIAL AND METHODS

Sampling

We sampled established old queens by carefully excavating whole nests using previously established procedures (e.g. Seppä 1994; Walin & Seppä 2001) at three sites in southern Finland, i) Savero (Kouvola) in 1992; ii) Antby (Raasepori) in 2004 and iii) TV/Leimann (Hanko). TV/Leimann population was sampled three times, first in 1994 for another study (Walin & Seppä 2001) and again in 2012 and 2014 (combined as TV/Leimann 2010s). Details of excavated populations and sampling are given in Table 1 and Appendix S1.

We also used pitfall-trap data of free-ranging wingless queens collected at two sites. In Musturi old-growth forest (Ruovesi, 1985), the sampling covered a 1.3 ha plot in a spruce-dominated old-growth reserve (Niemelä et al. 1989). In Multiharju (Seitseminen National Park, Ikaalinen, 1986), the sampling covered a 1.4 km transect consisting of a mixture of mostly old forest and forested mire types (Niemelä et al. 1992), with seven habitat types distinguished for the present study. For both sampling areas, the traps were under operation for most of the snow-free season, which we divided into three periods. Period I started in the early spring after the snowmelt and ended in mid-July before the

M. ruginodis nuptial flight. Period II covered the nuptial flight period, ending in mid-August and period III after that until late autumn. Details of sampled populations and sampling schemes are given in Table 1 and Appendix S1.

Finally, we also used queen data from two earlier excavation studies, one where the data were collected mostly from Scotland (hereafter Scotland, Brian & Brian, 1949) and another where the data were collected from southern England (Elmes, 1991) as a comparison to the Finnish data.

Morphometrics

Until 2004, we measured the head width of queens directly under a binocular to 0.02 mm accuracy. For the more recent excavations, we first photographed the sampled individuals with a Canon EOS 50D camera at a 25× magnification. Then we measured the head width of queens with the software ImageJ (Abràmoff *et al.*, 2004) to 0.02 mm accuracy. High repeatability of this method has been shown previously (Wolf & Seppä, 2016b). By optimising a bimodal fit to the queen-size distribution, head width of 1.065 mm was earlier determined as a cut-off to separate microgynes and macrogynes (Elmes, 1991). Later, this was shown to be in good accordance with a behavioural cut-off value based on the differential participation of queens of different size morphs in the nuptial flight in TV/Leimann population sampled in 2013 (Wolf & Seppä, 2016b). We divided nests to macrogynous, microgynous and mixed nests, or – for some analyses – assigned mixed nests based on their predominant morph type (median queen size in microgynes ≤ 1.06 mm).

Statistical analysis

In each population, we tested queen-size distributions for normality with Kolmogorov-Smirnov tests and for modality with Sarle's bimodality coefficient (BC, SAS Institute Inc, 1990). BC is calculated as a ratio of squared skewness and kurtosis of the size distribution, corrected for the sample size. The BC of a uniform distribution gets a value BC=0.56, while higher values indicate bimodality and lower ones unimodality (SAS Institute Inc, 1990). We tested whether the queen-size distributions in the TV/Leimann population sampled c. 20 years apart were similar with two-sample Kolmogorov-Smirnov-test. We also tested if queens from monogynous nests were larger than queens

from polygynous nests by fitting a linear mixed model to the data with monogyny/polygyny as a fixed factor and populations and nests nested into populations as random factors (Finnish populations only).

We used log-likelihood ratio tests (G test) to test the variation in queen number across populations by testing the difference in frequencies of queenless, monogynous and polygynous nests, and to test the variation in morph occurrence in macrogynous, microgynous and mixed nests across populations. We explored the association of size morphs with the level of polygyny (monogyny/polygyny) in the nests first in general by using log-likelihood ratio tests. Then we used binomial tests to assess if macrogynes and microgynes occurred more often than randomly in monogynous and polygynous nests, respectively, and if monogynous and polygynous nests had more often than randomly macrogynes and microgynes, respectively. Furthermore, we explored the association of the queen number with queen size by calculating Spearman rank correlation between queen number and mean queen size in nests (Finnish and S-English populations) and fitted a linear mixed model to the data to test the relationship between the proportion of microgynes and the number of queens in a nest was tested with the proportion microgynes as a fixed factor and populations as a random factor (Finnish populations only).

For the pitfall-trap data, we tested whether the free-ranging queens were distributed randomly among the sampling periods and habitat types (Multiharju only) using log-likelihood ratio test, calculating the expected occurrence rates based on the distribution of sampling effort among the habitat types and sampling periods. We also used log-likelihood ratio test to test whether the occurrence of macrogynes and microgynes was similar among the habitat types and sampling periods. We used SPSS 25.0 (IBM Corp. Released 2017) and Statistix 9.0 (Analytical Software 1985-2008) for the analyses.

Genetic analyses

The samples from 1990's (Savero, TV/Leimann 1994) were analysed with standard horizontal starch-gel electrophoresis. Genetic variation was resolved in four allozyme loci: α- glycerophosphate dehydrogenase-1 (aGpdh-1), peptidase (Pep, substrate:

glycyl-L-leucine), phosphoglycerate kinase (Pgk), and esterase-2 (Est-2, substrate: 4-methylumbelliferyl acetate) with protocols described in Seppä (1992).

We tested 21 microsatellite primers previously developed for other *Myrmica* species (Evans, 1993; Herbers & Mouser, 1998; Henrich *et al.*, 2003; Azuma *et al.*, 2005; Zeisset *et al.*, 2005). Twelve of these were polymorphic and amplified reliably in PCR in *M. ruginodis*, and we used them for samples from TV/Leimann 2010s. PCR products were analysed with an ABI 3730 sequencer and alleles scored using GeneMapper version 5 (Applied Biosystems). Details of DNA extraction, primer testing and PCR conditions are given in Appendix S2.

We tested queen recruitment pattern in three populations (Savero, TV/Leimann, 1994 and TV/Leimann 2010s) by estimating genetic relatedness among coexisting queens with the software RELATEDNESS 5.0.4. (Queller & Goodnight, 1989). We estimated the overall relatedness for the whole data and separately in macrogyne and microgyne nests and used t-tests to test whether relatedness estimates were significantly different from each other. In the analysis, we added the queens from monogynous nests in the data to calculate the background allele frequencies and jackknifed the estimates over loci and nests for standard errors.

RESULTS

Nest excavations and queen number

Between 32 and 132 entire nests were excavated in the six study populations (including the reference populations in the UK, Table 1). In *Myrmica*, a substantial proportion of nests are queenless (Elmes & Keller, 1993). In our populations, roughly one third of the nests were queenless, one third monogynous and one third polygynous, but the proportions of these categories varied widely and significantly across populations (Table 2; $G = 42.2$, $df = 8$, $P<0.001$). When regarding queenless nests as monogynous, the average estimated queen number in our populations varied between 1.03 and 3.91. [Previous genetic studies have shown that practically all queenless nests had been monogynous before losing their queen and the observed queen number (one vs. many) is a good proxy for the number of egg-layers in the nests (Seppä, 1994; Walin & Seppä, 2001)]. Disregarding the Antby population with only a single polygynous nest (3%), the

proportion of polygynous nests varied from 35% (Savero) to 60% (TV/Leimann, 1994), but the average queen number in polygynous nests varied only from four (S-England) to six (TV/Leimann, Table 2).

Queen-size distribution

Queen size was normally distributed only in one of our study populations. In the rest of the populations, queen-size distribution was non-normal and negatively skewed in six of eight populations and size distributions were uniform or bimodal (BC≥0.56) or nearly so in three populations (Fig. 1, 2, Table 3; Appendix S3, S4). In monogynous nests, queen sizes were non-normally distributed in half of the populations, but in all populations in polygynous nests (Table 2). Queen-size distributions in the TV/Leimann population sampled c. 20 years apart were similar (Table 3, Fig. 1, two-sample Kolmogorov-Smirnov-test, $P = 0.22$).

In general, queens in the monogynous nests were significantly larger than in the polygynous nests (linear mixed model, $F = 11.73$, $df = 90.88$, $P = 0.001$). The maximum queen size was similar in all populations (1.18-1.20 mm), but queen-size ranges varied across populations. The size range was largest in TV/Leimann and Multiharju (minimum queen size 0.84 mm) and smallest in predominantly macrogynous populations (Antby, Musturi, Savero; minimum 0.98 mm). In both TV/Leimann data sets, the smallest queens were sampled from two highly polygynous nests and constituted a majority of queens in those nests (Appendix S3).

Queen-number association with the size morphs

The frequency of microgyne queens in excavated populations ranged from c. 25% to c. 65% (Table 2). The frequency of mixed nests (polygynous nests that had queens of both size morphs) ranged from zero to 50% of the polygynous nests (Table 2), and the proportions of nest types (pure macrogyne nests, pure microgyne nests, mixed nests) varied significantly across populations (Table 2, $G = 26.82$, $df = 8$, $P < 0.001$). In the pitfall-trap data sets, 10 and 30% of queens were microgynes.

292 At the population level, queen morphs were not distributed randomly to monogynous
293 and polygynous nests (Table 2, Finnish populations combined, G = 14.4, df = 1, P <
294 0.001). Microgynes occurred significantly more often in polygynous than monogynous
295 nests (binomial test, P < 0.001), but there was no significant difference in macrogyne
296 occurrence in monogynous and polygynous nests (binomial test, P = 0.072).
297 Furthermore, monogynous nests had significantly more often macrogynes than
298 microgynes (binomial test, P < 0.001), but there was no significant difference in morph
299 occurrence in polygynous nests (binomial test, P = 0.082).

300 At the nest level, we found a significant positive association when comparing the
301 proportion of microgynes with the number of queens (Finnish populations combined,
302 linear mixed model, F = 9.54, df = 129.00, P = 0.002). Queen number and mean queen
303 size in the nests were also significantly negatively correlated in both Finnish and S-
304 English populations (Spearman rank correlation; all Finnish samples combined: r_s = -
305 0.39, P < 0.001, N = 131; S-England: r_s = -0.55, P< 0.001, N = 79).

306

307 **Distribution of free-ranging wingless queens within season and across habitats**

308 All queens sampled by pitfall trapping were wingless. In both pitfall data sets, the
309 occurrence of *M. ruginodis* queens across the three sampling periods (before, during and
310 after the nuptial flight) was non-random (Fig. 3, Appendix S4, Musturi: G = 17.5, df =
311 2, p < 0.001; Multiharju: G = 8.2, df = 2, p < 0.05). Period before the nuptial flight
312 (Period I) in both Musturi and Multiharju had the highest cell contributions (deficiency
313 of gynes in Musturi, excess of gynes in Multiharju) to the test statistics. Distribution of
314 macrogynes and microgynes was similar across the sampling periods in both data sets
315 (Fig. 3, Appendix S4, Musturi: G = 0.87, df = 2, P = 0.65; Multiharju: G = 0.65, df = 2,
316 P = 0.69).

317 In Multiharju, there was a significant difference in the occurrence rate of queens across
318 habitats (Fig. 4, G = 34.2, df = 6, P < 0.001), with old spruce-pine mire (habitat type 5)
319 showing the largest cell contribution (excess of queens compared to the random
320 expectation) and old spruce-dominated mesic heath forest (habitat type 4) the next
321 largest contribution (deficiency of queens) to the test statistics. However, the queen-

morph distributions across the habitat types did not differ significantly from each other (Fig. 4, Appendix S4, G = 10.7, df = 6, P = 0.099).

Genetic relatedness among queen nest mates

Average relatedness among queen nest mates was similar in all three populations where genetic data were available (Table 4, Savero vs. TV/Leimann 1994, t-test, t = 0.85, df = 39, P = 0.40; statistical differences to TV/Leimann 2010s population were not tested because the genetic marker used was different). When estimated separately for polygynous macrogyne and microgyne nests, relatedness in macrogyne nests was significantly lower than relatedness in microgyne nests in one population (TV/Leimann 1994: t = 4.10, df = 19, P< 0.001), but not in two others (Savero: t = 0.26, df = 18, P = 0.80; TV/Leimann 2010s: t = 0.84, df = 32, P = 0.41).

DISCUSSION

Alternative reproductive strategies associated with intraspecific size variation are common (e.g. Eberhard, 1980; Thornhill & Alcock, 1983; Gross, 1985, 1996; Crespi, 1988; Danforth, 1991) and in ants they are usually manifested only in females (Heinze & Tsuji, 1995; Peeters & Ito, 2001). Our work shows that the queen-size morphs in the ant *Myrmica ruginodis* are associated with key life-history traits, such as queen number, dispersal and queen recruitment, but the association is not universal and particularly the number of queens in the nests can be extremely flexible.

Large variation in queen number within and across study populations is in line with previous studies on many facultatively polygynous ants, including other *Myrmica* sp. (e.g. Elmes & Keller, 1993; Crozier & Pamilo, 1996; Pamilo et al 1997; Sundström *et al.*, 2005). Queen number can also vary temporally within single *Myrmica* populations (Elmes, 1987; Elmes & Petal, 1990) due to the irregular gyne production in the nests (Elmes, 1973; Elmes & Petal, 1990). The occurrence of queenless nests (up to 50%) is due to the fast queen turnover in *Myrmica*, which often leaves nests queenless after the demise of the queen(s), particularly when the queen number is low (Seppä, 1994, Evans,

1996). This also emphasises queen number as an unusually flexible trait in *Myrmica* ants (Elmes & Keller, 1993).

Queen morph – queen number association

Queen size was normally distributed only in one of our study populations (Antby), which results from this largely monogynous population being dominated by a single queen morph (macrogynes). In the rest of the populations, the actual size ranges and the non-normal, negatively skewed or bimodal queen-size distributions suggest that they are a mixture of both queen morphs (Figures 1-2, Appendices S3-4), and that the frequency of the morphs varies widely across the populations. Considering that previous work has described *M. ruginodis* as a size-dimorphic species (Brian & Brian, 1949, 1955; Elmes, 1991), finding a strongly macrogyne-dominated population with a normal queen-size distribution is unexpected. Morph frequencies and social structure vary extensively among nests and among (sub)populations also in two other queen-size dimorphic ants, *Temnothorax rugatulus* (Rüppell *et al.*, 1998, 2001) and *Ectatomma ruidum* (Chantal Poteaux, pers. communication).

Queen number was associated with the occurrence of the size morphs in *M. ruginodis*, but not in all populations. The proportion of microgynes and the number of queens in the nest were positively correlated and the average queen size in nest decreased with increasing queen number, which is common in many polygynous ants (e.g. *Formica truncorum*, Sundström, 1995; *F. selysi*, Meunier & Chapuisat, 2009). Furthermore, macrogynes were associated with monogyny and microgynes with polygyny more often than randomly, but all associations were not significant. All these indicate that there is at least an indirect link in *M. ruginodis* between the level of polygyny and the frequency of microgynes. However, in one study population (Savero), the majority of macrogynes and microgynes were found in polygynous and monogynous nests, respectively (Table 2), which is reversed compared to the expected. This suggests that queen number as a plastic trait is also affected by other factors than morph frequencies in *M. ruginodis*. Definite conclusions based on Savero population are difficult to draw, however, because the number of microgynes found is small.

Queen dispersal and recruitment

Seasonal distribution of free-ranging wingless queens caught by pitfall-trapping was not random within the study populations, but the distributions of macrogynes and microgynes caught in the same populations were similar. Thus, our result does not support our prediction of different dispersal patterns in populations with different proportions of microgynes. In the macrogyne dominated Musturi population, we expected to find an excess of free-ranging wingless queens directly after the nuptial flight (period III), but we only found a deficiency of queens during period I (from the start of the season until nuptial flights; Fig. 3, Appendix S4). As microgyne queens are expected to move outside their nests more actively than macrogynes due to commonly occurring dependent colony founding in polygynous ants, we expected to find a more even distribution of free-ranging queens across the season in Multiharju with a relatively large proportion of microgynes. Instead, there was an excess of queens caught from Multiharju only during period I (Fig. 3, Appendix S4).

In accordance with previous results (Seppä, 1994), coexisting queens are related in polygynous nests on average, which indicates that nests must have recruited their own daughters as new queens. Previous dispersal experiments in the TV/Leimann population revealed that most *M. ruginodis* microgynes do not join mating swarms, i.e. they do not disperse by flight (Wolf & Seppä, 2016b). Instead, they probably mate in the vicinity of their natal nest and high relatedness among coexisting microgyne queens corroborates that many microgynes are recruited back to their natal nest. Our relatedness results show that the recruitment process in macrogynes is at least partly different, as coexisting macrogyne queens were not related at all in TV/Leimann 1994 data. However, relatedness among coexisting macrogynes in polygynous nests is difficult to predict since most macrogynes join a mating swarm (Wolf & Seppä 2016b) and presumably later found new monogynous colonies independently.

If a macrogyne queen first joins a nuptial flight, she has three alternatives that would result in high queen relatedness in a polygynous nest: she can i) return to her natal nest after leaving the mating swarm; ii) found a new nest independently with related queens or iii) seek adoption in a random nest simultaneously with other related queens. All these alternatives are unlikely, however. *M. ruginodis* mating flights are rather extensive

and attract winged sexuals from a large number of nests from a relatively large area (Brian & Brian, 1949; Elmes, 1991; Wolf & Seppä, 2016b, unpublished observations). Orientation of flying *M. ruginodis* queens has not been studied, but returning to the natal nest from a distant nuptial flight site seems unlikely. Furthermore, pleometrosis, independent nest founding by a group of queens, has never been reported in *M. ruginodis* and related queens seeking entrance to the same non-natal nest must be rare.

However, queen relatedness patterns in both microgyne and macrogyne nests suggests that not all queens follow the queen-dispersal dichotomy suggested – long-range dispersal of macrogynes by flight and local dispersal of microgynes by foot (Wolf & Seppä, 2016b), and that part can be large enough to change the relatedness regime in the nests. On one hand, positive relatedness in macrogyne nests must be caused by some macrogynes forgoing the nuptial flight and joining their natal nests afterwards. On the other hand, relatedness is rather variable across microgyne nests in TV/Leimann 2010s and Savero populations based on the large standard errors (Table 4), suggesting that nests recruit both their own daughters and unrelated queens.

Habitat association of free-ranging *M. ruginodis* queens

Our results on the distribution of free-ranging wingless queens showed that queens sampled were non-randomly distributed across different habitat types, but that there was no difference in how queen morphs were distributed. Thus, our results do not support the hypothesis that *M. ruginodis* microgynes specialise in stable habitats in successional boreal forests.

Apart from habitat stability, differences in microclimate, such as shadiness, moisture and presence of competitors may have a strong influence on the morph occurrence. Although the moor sites in the UK studied by Elmes (1978, 1991) ranged from dry to very wet, the common feature and major difference to the Finnish sites is presumably the lack of shadiness (absence of trees) in English populations, as most of the English sampling sites were covered by lower plants only (grass, herbs, dwarf shrubs etc). This lower vegetation will generally offer more sun-exposed habitats, especially when grazing animals maintain them relatively open (Elmes, 1978). *Myrmica ruginodis* is the least thermophile of all the *Myrmica* species (Radchenko & Elmes, 2010) and capable

of inhabiting also closed-canopy forests (e.g. Punttila *et al.*, 1991, 1994, 2016, Niemelä et al. 1996). Most of our study areas were mesic forests and thus generally rather shady. Thus, a combination of habitat stability and amicable microclimate (lack of closed tree canopy) could in general explain the higher occurrence of the microgyne morph in the UK (c. 70%) compared to the North-European boreal forests (c. 10-50%). In the former habitat type, selection for good dispersal ability is relaxed, which is expected to favour the less-dispersive microgyne morph. This could be tested by studying spatial population structure in the two areas.

Boreal mesic heath forests undergo a continuous successional change and cannot be described as stable, with perhaps the slowly changing old-growth stage as the only exception (Kuuluvainen, 2009; Kuuluvainen & Aakala, 2011). Thus, habitat stability as such cannot be used as a direct predictor of microgyne occurrence in boreal forests, as suggested by Brian & Brian (1949) for the moor habitats in the UK. An earlier genetic study on *M. ruginodis* in successional boreal forests also contrasts the habitat stability hypothesis (Seppä *et al.*, 1995): On one hand, worker nestmate relatedness, a proxy for the level of polygyny and thus for the frequency of microgyne morph, was lowest in the young forest patches following disturbance (clearcutting, sometimes combined with prescribed burning). On the other hand, the population sampled from an old-growth forest that could be considered the most stable habitat compared to the rest of the populations is practically monogynous, suggesting the absence of microgynes (Seppä *et al.*, 1995).

Conclusions

Our study, together with our previous results (Wolf & Seppä, 2016), partly confirms the association of queen-size morphs with key life-history traits in the ant *Myrmica ruginodis*, but the association is not straightforward and universal, with individual populations showing even reversed associations. Particularly the queen number is an unusually plastic trait in *Myrmica* ants (e.g. Elmes & Keller 1993), which evidently applies also separately to the size morphs of *M. ruginodis*. Indeed, the conventional dichotomy to monogynous and polygynous ants with associated distinct life-history traits seems as a simplification. In reality, many ants are facultatively polygynous and

life-history traits are neither simple binary traits nor associated with only a single social colony structure. Especially, a critical distinction in queen number concerning queen dispersal and subsequent nest-founding patterns does not seem to be the transition from monogyny to polygyny, but from facultatively to obligatory polygyny (Sundström *et al.*, 2005; Boomsma *et al.*, 2014).

ACKNOWLEDGEMENTS

This work was funded by the Academy of Finland (Centre of Excellence in Biological Interactions, grant no. 251337 and 284666). We thank Heini Ali-Kovero, Yrjö Haila, Eero Halme, Markus Haveri, Jari Niemelä, Cia Olsson, Timo Pajunen, Harri Tukia and Laura Walin for help in the field and the lab, Sanja Hakala for help with statistical analyses, and Heikki Helanterä, David Nash, Pekka Pamilo and anonymous reviewers for comments on the manuscript.

CONTRIBUTION OF AUTHORS

All authors contributed to the project design, data collection and analysis, and paper writing.

SUPPORTING INFORMATION

Appendix S1: Sampling Scheme

Appendix S2: Laboratory protocols for DNA extraction and DNA microsatellite analysis

Appendix S3: Queen size data, excavated nests

Appendix S4: Queen size data, pitfall data

REFERENCES

Abràmoff, M.D., Magalhães, P.J. & Ram SJ (2004) Image processing with ImageJ. *Biophotonics International*, **11**, 36-42.

Analytical Software (1985-2008) Statistix 9. Tallahassee FL, USA.

Anholt, B.R. (1990) Size-biased dispersal prior to breeding in a damselfly, *Oecologia* **83**, 385-387. doi: 10.1007/BF00317564

Azuma, N., Takahashi, J., Kikuchi, T., Yoshimura, M., Onoyama, K. & Higashi S (2005) Microsatellite loci for *Myrmica kotokui* and their application in some congeneric ant species distributed in northern Japan, *Molecular Ecology Notes* **5**, 118-120. doi: 10.1111/j.1471-8286.2004.00849.x

Barbraud, C., Johnson, A.R. & Bertault, G. (2003) Phenotypic correlates of post-fledging dispersal in a populations of greater flamingos: the importance of body condition. Journal of Animal Ecology, **72**, 246-257. doi: 10.1046/j.1365-2656.2003.00695.x

Boomsma, J.J., Huszár, D.B. & Pedersen, J.S. (2014) The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. *Animal Behavior*, **92**, 241-252.

Brian, M.V. & Brian, A.D. (1949) Observations on the taxonomy of the ants *Myrmica rubra* L. and *M. laevinodis* Nylander (Hymenoptera: Formicidae). *Transactions of the Royal Entomological Society of London*, **100**, 393-409. doi: 10.1111/j.1365-2311.1949.tb01423.x

Brian, M.V. & Brian, A.D. (1955) On the two forms macrogyna and microgyna of the ant *Myrmica rubra* L. *Evolution*, **9**, 280-290. doi 10.2307/2405649

Cajander, A.K. (1949) Forest types and their significance. *Acta Forestalia Fennica*, **56**, 21-38

Collingwood, C.A. (1979) *The Formicidae (Hymenoptera) of Fennoscandia and Denmark*. Scandinavian Science Press Ltd, Klampenborg, Denmark.

Crespi, B.J. (1988) Adaptation, compromise, and constraint: the development, morphometrics, and behavioral basis of a fighter-flier polymorphism in male *Hoplothrips karnyi* (Insecta: Thysanoptera). *Behavioral Ecology and Sociobiology*, **23**, 93-104. doi: 10.1007/bf00299892

530 Crozier, R.H. & Pamilo, P. (1996) *Evolution of social insect colonies: sex allocation and*
531 *kin selection*. Oxford University Press, Oxford, U.K.
532 Czechowski, W., Radchenko, A., Czechowska, W. & Vepsäläinen, K. (2012) *The ants of*
533 *Poland with reference to the myrmecofauna of Europe*. Fauna Poloniae, *4* , 1-496.
534 Danforth, B.N. (1991) The morphology and behavior of dimorphic males in *Perdita*
535 *portalis* (Hymenoptera: Andrenidae). *Behavioral Ecology and Sociobiology*, **29**, 235-
536 247. doi: 10.1007/bf00163980
537 Derr, J.A., Alden, B. & Dingle, H. (1981) Insect life histories in relation to migration,
538 body size, and host plant array: a comparative study of *Dysdercus*. *Journal of Animal*
539 *Ecology*, **50**, 181-194 doi: 10.2307/4039
540 Dingle, H., Blakley, N.R. & Miller, E.R. (1980) Variation in body size and flight
541 performance in milkweed bugs (*Oncopeltus*). *Evolution*, **34**, 371-385. doi:
542 10.2307/2407400
543 Eberhard, W.G. (1980) Horned beetles. *Scientific American*, **242**, 166-182. doi:
544 10.1038/scientificamerican0380-166
545 Elmes, G.W. (1973) Observations on the density of queens in natural colonies of
546 *Myrmica rubra* L. (Hymenoptera: Formicidae). *Journal of Animal Ecology, 42*, 761-
547 771. doi: 10.2307/3136
548 Elmes, G.W. (1976) Some observations on the microgyne form of *Myrmica rubra* L.
549 (Hymenoptera, Formicidae). *Insect Sociaux*, **21**, 3-22 doi: 10.1007/BF02283902
550 Elmes, G.W. (1978) Populations of *Myrmica* (Formicidae) living on different types of
551 *Calluna* moorland – a semi-natural habitat of Southern England. *Memorabilia*
552 *Zoologica*, **29**, 41-60
553 Elmes, G.W. (1987) Temporal variation in colony populations of the ant *Myrmica*
554 *sulcinodis*: I. Changes in queen number, worker number and spring production.
555 *Journal of Animal Ecology*, **56**, 559-571. doi: 10.2307/5068
556 Elmes, G.W. (1991) Mating strategy and isolation between the two forms, macrogyna
557 and microgyna, of *Myrmica ruginodis* (Hym. Formicidae). *Ecological Entomology*,
558 **16**, 411-423. doi: 10.1111/j.1365-2311.1991.tb00234.x
559 Elmes GW, Petal J (1990) Queen number as an adaptable trait: evidence from wild
560 populations of two red ant species (genus *Myrmica*). *Journal of Animal Ecology*
561 p.675-690 doi: 10.2307/4888

562 Elmes, G.W. & Keller, L. (1993) Distribution and ecology of queen number in ants of
563 the genus *Myrmica*. *Queen number and Sociality in Insects* (ed. by L. Keller), pp.
564 294-307. Oxford University Press, Oxford, U.K.
565 Evans, J.D. (1993) Parentage analyses in ant colonies using simple sequence repeat loci.
566 *Molecular Ecology*, 2:393-397 doi: 10.1111/j.1365-294x.1993.tb00032.x
567 Evans, J.D. (1996) Queen longevity, queen adoption, and posthumous indirect fitness in
568 the facultatively polygynous ant *Myrmica tahoensis*. *Behavioral Ecology and*
569 *Sociobiology* **39**, 275-284. doi: 10.1007/s002650050290
570 Gonzaga, M.D.O. & Vasconcellos-Neto, J. (2001) Female body size, fecundity
571 parameters and foundation of new colonies in *Anelosimus jabaquara* (Araneae,
572 Theridiidae). *Insectes Sociaux*, **48**, 94-100. doi: 10.1007/pl00001765
573 Gross, M.R. (1985) Disruptive selection for alternative life histories in salmon. *Nature*,
574 **313**, 47-48 doi: 10.1038/313047a0
575 Gross, M.R. (1996) Alternative reproductive strategies and tactics: diversity within
576 sexes. *Trends in Ecology and Evolution*, **11**, 92-98. doi: 10.1016/0169-
577 5347(96)81050-0
578 Greenwood, P.J. (1980) Mating systems, philopatry and dispersal in birds and mammals.
579 *Animal Behavior*, **28**, 1140-1162. doi: 10.1016/s0003-3472(80)80103-5
580 Gundersen, G., Andreassen, H.P. & Ims, R.A. (2002) Individual and population level
581 determinants of immigration success on local habitat patches: an experimental
582 approach. *Ecology Letters*, **5**, 294-301. doi: 10.1046/j.1461-0248.2002.00320.x
583 Hanski, I., Peltonen, A. & Kaski, L. (1991) Natal dispersal and social dominance in the
584 common shrew *Sorex araneus*. *Oikos*, **62**, 48-58. doi: 10.2307/3545445
585 Heinze, J. & Hölldobler, B. (1993) Queen polymorphism in an Australian weaver ant,
586 *Polyrachis* cf. *doddi*. *Psyche*, **100**, 83-92. doi: 10.1155/1993/47808
587 Heinze, J. & Keller, L. (2000) Alternative reproductive strategies: a queen perspective in
588 ants. *Trends in Ecology and Evolution*, **15**, 508-512. doi: 10.1016/s0169-
589 5347(00)01995-9
590 Heinze, J. & Tsuji, K. (1995) Ant reproductive strategies. *Researches on Population*
591 *Ecology*, *37*, 135-149. doi: 10.1007/bf02515814

592 Henrich, K.O., Sander, A.C., Wolters, V. & Dauber, J. (2003) Isolation and
593 characterization of microsatellite loci in the ant *Myrmica scabrinodis*. *Molecular*
594 *Ecology Notes*, **3**, 304-306. doi: 10.1046/j.1471-8286.2003.00433.x
595 Herbers, J.M. (1986) Nest site limitation and facultative polygyny in the ant *Leptothorax*
596 *longispinosus*. *Behavioral Ecology and Sociobiology*, **19**, 115-122. doi:
597 10.1007/bf00299946
598 Herbers, J.M. (1993) Ecological determinants of queen number in ants. *Queen number*
599 *and Sociality in Insects* (ed. by L. Keller), pp. 262-293. Oxford University Press,
600 Oxford, U.K.
601 Herbers, J.M. & Mouser, R.L. (1998) Microsatellite DNA markers reveal details of
602 social structure in forest ants. *Molecular Ecology*, **7**, 299-306. doi: 10.1046/j.1365-
603 294x.1998.00349.x
604 Hölldobler, B. & Wilson, E.O. (1977) The number of queens: an important trait in ant
605 evolution. *Naturwissenschaften*, **64**, 8-15. doi: 10.1007/bf00439886
606 Hölldobler, B. & Wilson, E.O. (1990) *The ants*. Springer-Verlag, Berlin.
607 IBM Corp. Released 2017. *IBM SPSS Statistics for Windows, Version 25.0*. Armonk,
608 NY: IBM Corp.
609 Keller, L. (1995) Social life: the paradox of multiple-queen colonies. *Trends in Ecology*
610 *and Evolution*, **10**, 355-360. doi: 10.1016/s0169-5347(00)89133-8
611 Kuuluvainen, T. (2009) Forest management and biodiversity conservation based on
612 natural ecosystem dynamics in Northern Europe: The complexity challenge. **Ambio**,
613 **38**, 309-315. doi: 10.1579/08-a-490.1
614 Kuuluvainen, T. & Aakala, T. (2011) Natural forest dynamics in boreal Fennoscandia: a
615 review and classification. *Silva Fennica*, **45**, 823-841. doi: 10.14214/sf.73
616 Lachaud, J.P., Cadena, A., Schatz, B., Pérez-Lachaud, G. & Ibarra-Núñez, G. (1999)
617 Queen dimorphism and reproductive capacity in the ponerine ant, *Ectatomma ruidum*
618 Roger. *Oecologia*, **120**, 515-523. doi: 10.1007/s004420050885
619 Lawrence, W.S. (1987) Dispersal: an alternative mating tactic conditional on sex ratio
620 and body size. *Behavioral Ecology and Sociobiology*, **21**, 367-373. doi:
621 10.1007/bf00299931

622 Léna, J.P., Clobert, J., De Fraipont, M., Lecomte, J. & Guyot, G. (1998) The relative
623 influence of density and kinship on dispersal in the common lizard. *Behavioral*
624 *Ecology*, **9**, 500-507. doi: 10.1093/beheco/9.5.500
625 Lenoir, J.C., Lachaud, J.P., Nettel, A., Fresneau, D. & Poteaux, C. (2011) The role of
626 microgynes in the reproductive strategy of the neotropical ant *Ectatomma ruidum*.
627 *Naturwissenschaften*, **98**, 347-356. doi: 10.1007/s00114-011-0774-3
628 Leppänen, J., Seppä, P., Vepsäläinen, K. & Savolainen, R. (2015) Genetic divergence
629 between the sympatric queen morphs in the ant *Myrmica rubra*. *Molecular Ecology*
630 **24**, 2463-2476, doi:10.1111/mec.13170
631 McInnes, D.A. & Tschinkel, W.R. (1995) Queen dimorphism and reproductive strategies
632 in the fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Behavioral Ecology*
633 *and Sociobiology*, **36**, 367-375. doi: 10.1007/bf00177332
634 Meunier, J. & Chapuisat, M. (2009) The determinants of queen size in a socially
635 polymorphic ant. *Journal of Evolutionary Biology*, **22**, 1906-1913. doi:
636 10.1111/j.1420-9101.2009.01805.x
637 Niemelä, J., Haila, Y., Halme, E., Pajunen, T. & Punttila, P. (1989) The annual activity
638 cycle of carabid beetles in the southern Finnish taiga. *Annales Zoologici Fennici*, **26**,
639 35-41.
640 Niemelä, J., Haila, Y., Halme, E., Pajunen, T. & Punttila, P. (1992) Small-scale
641 heterogeneity in the spatial distribution of carabid beetles in the southern Finnish
642 taiga. *Journal of Biogeography*, **19**, 173-181. doi: 10.2307/2845503
643 Niemelä, J., Haila, Y. & Punttila, P. (1996) The importance of small-scale heterogeneity
644 in boreal forests: variation in diversity in forest-floor invertebrates across the
645 succession gradient. *Ecography*, 19: 352-368.
646 O'Riain, M.J., Jarvis, J.U.M. & Faulkes, C.G. (1996) A dispersive morph in the naked
647 mole-rat. *Nature*, **380**, 619-621. doi: 10.1038/380619a0
648 Pamilo, P., Gertsch, P., Thorén, P. & Seppä, P. (1997) Molecular population genetics in
649 social insects. *Annual Review of Ecology and Systematics* **28**, 1-25.
650 Passera, L. & Keller, L. (1990) Loss of mating flight and shift in the pattern of
651 carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). *Journal of*
652 *Comparative Physiology B*, **160**, 207-211. doi: 10.1007/bf00300955

653 Peeters, C. & Ito, F. (2001) Colony dispersal and the evolution of queen morphology in
654 social Hymenoptera. *Annual Review of Entomology*, **46**, 601-630. doi:
655 10.1146/annurev.ento.46.1.601
656 Punttila, P., Haila, Y., Pajunen, T. & Tukia, H. (1991) Colonisation of clearcut forests by
657 ants in the southern Finnish taiga: a quantitative survey. *Oikos*, **61**, 250-262. doi:
658 10.2307/3545343
659 Punttila, P., Haila, Y., Niemelä, J. & Pajunen, T. (1994) Ant communities in fragments
660 of old-growth taiga and managed surroundings. *Annales Zoologici Fennici*, **31**, 131-
661 144.
662 Punttila, P., Autio, O., Kotiaho, J.S., Kotze, D.J., Loukola, O.J., Noreika, N. &
663 Vepsäläinen, K. (2016) The effects of drainage and restoration of pine mires on
664 habitat structure, vegetation and ants. *Silva Fennica*, **50**, 1462. doi: 10.14214/sf.1462
665 Queller, D.C. & Goodnight, K.F. (1989) Estimating relatedness using genetic markers.
666 *Evolution*, **43**, 258-275. doi: 10.2307/2409206
667 Radchenko, A.G. & Elmes, G.W. (2010) Myrmica *ants (Hymenoptera, Formicidae) of*
668 *the old world*. Natura Optima Dux Foundation, Warsaw, Poland.
669 Roff, D.A. (1992) *The evolution of life histories: theory and analysis*. Chapman and
670 Hall, New York, U.S.A.
671 Rosengren, R. & Pamilo, P. (1983) Evolution of polygyny and polydomy in mound-
672 building *Formica* ants. *Acta Entomologica Fennica*, **42**, 65-77.
673 Rüppell, O. & Heinze, J. (1999) Alternative reproductive tactics in females: the case of
674 size polymorphism in winged ant queens. *Insectes Sociaux*, **46**, 6-17. doi:
675 10.1007/s000400050106
676 Rüppell, O., Heinze, J. & Hölldobler, B. (1998) Size-dimorphism in the queens of the
677 North American ant *Leptothorax rugatulus* (Emery). *Insectes Sociaux*, **45**, 67-77.
678 doi: 10.1007/s000400050069
679 Rüppell, O., Heinze, J. & Hölldobler, B (2001) Alternative reproductive tactics in the
680 queen-size-dimorphic ant *Leptothorax rugatulus* (Emery) and their consequences for
681 genetic population structure. *Behavioral Ecology and Sociobiology*, **50**, 189-197. doi:
682 10.1007/s002650100359
683 SAS Institute Inc. (1990) SAS/STAT User's Guide, Version 6, 4th Edn.

684 Seifert, B. (1993) Taxonomic description of *Myrmica microrubra* n. sp. – a social
685 parasitic ant so far known as the microgyne of *Myrmica rubra* (L.). *Abhandlungen*
686 *und Berichte des Naturkunde Museums Görlitz* **67**, 9-12.
687 Seppä, P. (1992) Genetic relatedness of worker nestmates in *Myrmica ruginodis*
688 (Hymenoptera: Formicidae) populations. *Behavioral Ecology and Sociobiology*, **30**,
689 253-260. doi: 10.1007/bf00166710
690 Seppä, P. (1994) Sociogenetic organization of the ants *Myrmica ruginodis* and *Myrmica*
691 *lobicornis*: number, relatedness and longevity of reproducing individuals. *Journal of*
692 *Evolutionary Biology*, **7**, 71-95. doi: 10.1046/j.1420-9101.1994.7010071.x
693 Seppä, P., Sundström, L. & Punttila, P. (1995) Facultative polygyny and habitat
694 succession in boreal ants. *Biological Journal of Linnean Society*, **56**, 533-551. doi:
695 10.1111/j.1095-8312.1995.tb01109.x
696 Stearns, S.C. (1992) *The evolution of life histories*. Oxford University Press, New York.
697 Stille, M. (1996) Queen/worker thorax volume ratios and nest-founding strategies in
698 ants. *Oecologia*, **105**, 87-93. doi: 10.1007/bf00328795
699 Sundström, L. (1995) Dispersal polymorphism and physiological condition of males and
700 females in the ant *Formica truncorum*. *Behavioral Ecology*, **6**, 132-139. doi:
701 10.1093/beheco/6.2.132
702 Sundström, L., Seppä, P. &Pamilo P (2005) Genetic population structure and dispersal
703 patterns in *Formica* ants − a review. *Annales Zoologici Fennici*, **42**, 163-177.
704 Thornhill, R. & Alcock, .J (1983) *The evolution of insect mating systems*. Harvard
705 University Press, Cambridge, Mass.
706 Walin, L. & Seppä, P. (2001) Resource allocation in the red ant *Myrmica ruginodis* − an
707 interplay of genetics and ecology. *Journal of Evolutionary Biology*, **14**, 694-707. doi:
708 10.1046/j.1420-9101.2001.00332.x
709 Wheeler, D.E. & Martinez, T. (1995) Storage proteins in ants (Hymenoptera:
710 Formicidae). *Comparative Biochemistry and Physiology B – Biochemistry &*
711 *Molecular Biology*, **112**, 15-19. doi: 10.1016/0305-0491(95)00035-7
712 Wolf, J.I. & Seppä, P. (2016a) Queen size dimorphism in social insects. *Insectes*
713 *Sociaux*, **63**, 25-38. doi: 10.1007/s00040-015-0445-z
714 Wolf, J.I. & Seppä, P. (2016b) Dispersal and mating in a size dimorphic ant. *Behavioral*
715 *Ecology and Sociobiology*, **70**, 1267-1276. doi: 10.1007/s00265-016-2135-x

716 Wolf, J.I. 2016 *Genetic and behavioural divergence of queen size morphs in the red ant*
717 Myrmica ruginodis. PhD dissertation, University of Helsinki, Helsinki, Finland
718 URN:ISBN:978-951-51-2454-8
719 Zeisset, I., Ebsen, J.R. & Boomsma, J.J. (2005) Dinucleotide microsatellite DNA loci
720 from the ant *Myrmica scabrinodis*. *Molecular Ecology Notes*, **5**, 163-164. doi:
721 10.1111/j.1471-8286.2004.00871.x